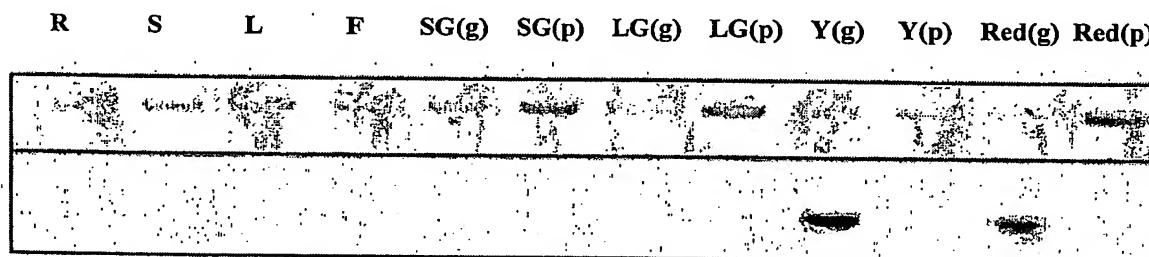


5 **Figure 1:** Northern blot analysis of the expression of the cysteine proteinase (CcCP1) gene in different tissues of *Coffea arabica*.

10



15 **Figure 2:** Northern blot analysis of the expression of the Cysteine proteinase CcCP-1 gene in different tissues of *Coffea arabica*.

Figure 2A: Alignment of the full sequence of the protein encoded by CcCP-1 cDNA with other full-length cysteine proteinases available in the NCBI database.



5 **Figure 3:** Northern blot analysis of the expression of the cysteine proteinase inhibitor (CcCPI-1) gene in different tissues of *Coffea arabica*.

10

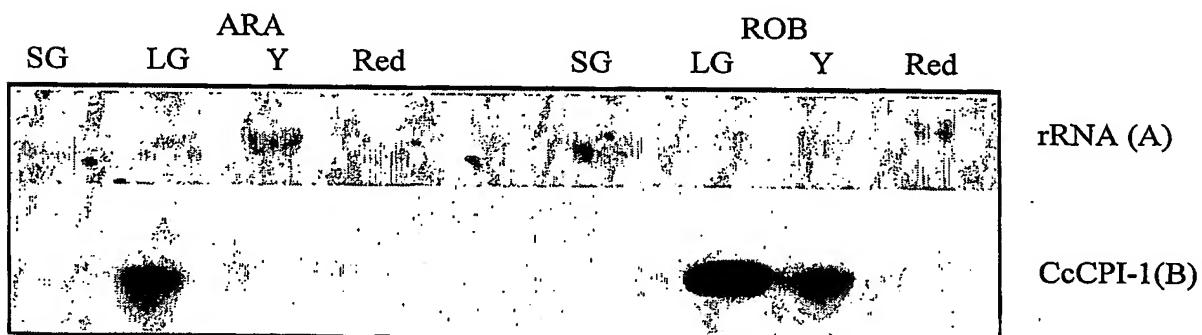


Figure 4: Northern blot analysis of the expression of the cysteine proteinase inhibitor gene (CcCPI-1) at different cherry development stages for *Coffea arabica* (ARA) and *Coffea canephora* (ROB).

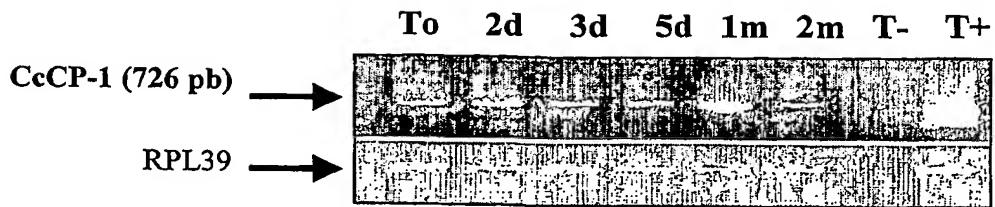


Figure 5. RT-PCR analysis of the expression of *CcCP-1* during *Coffea arabica* grain germination.

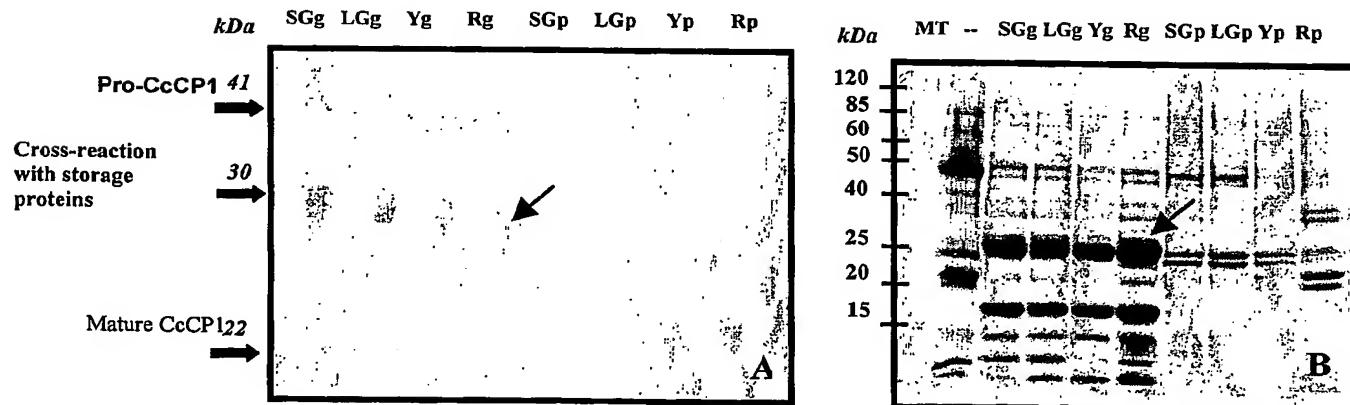
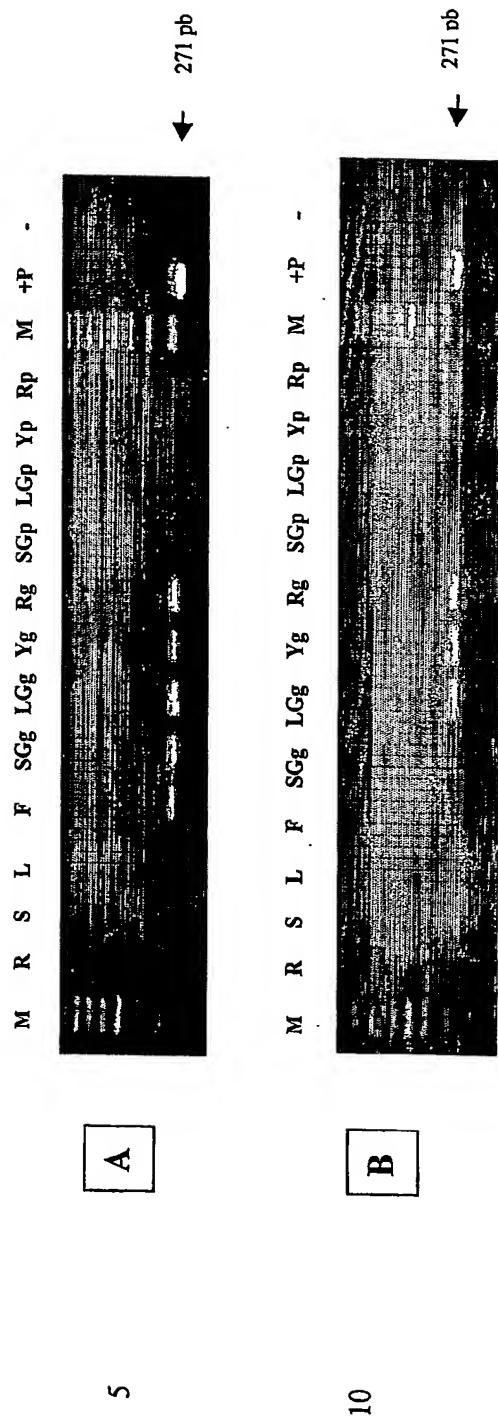


Figure 6: Western-blot analysis of the expression of CcCP1 protein (A).

Figure 6A: Optimal alignment of the complete protein encoded by CcCPI-1 cDNA with other homologous full-length cysteine proteinases available in the NCBI.



15 - Figure 7: RT-PCR analysis of the expression of CcCPI-1 gene in different tissues of *Coffea arabica* CCCA2 (A) and *Coffea robusta* FRT-32 (B).

	CcCPI-2																					
1	M	A	K	V	G	G	I	S	E	S	K	G	N	-	E	N	S	L	E	I	E	
1	M	A	T	I	G	G	I	K	Q	V	E	G	S	-	A	N	S	L	E	V	E	
1	M	A	T	V	G	G	I	K	D	S	G	G	S	S	A	N	S	L	E	I	D	
1	M	A	T	L	G	G	I	K	E	V	E	S	-	A	N	S	V	E	I	D	N	
40	F	Q	K	V	I	N	S	K	E	Q	V	V	A	G	T	V	Y	Y	L	T	I	
40	F	S	K	V	V	N	T	K	E	Q	V	V	A	G	T	M	Y	Y	I	T	L	
41	F	Q	R	V	V	N	T	K	E	Q	V	V	A	G	T	I	Y	Y	I	T	L	
40	F	K	R	V	V	S	T	K	Q	V	V	A	G	T	M	Y	Y	I	T	L	F	
80	W	L	N	F	K	E	V	Q	E	F	K	P	A	A	G	D	T	S	A	-		
80	W	M	N	F	K	Q	V	Q	E	F	K	L	L	G	D	Q	G	S	T	S		
81	W	V	N	F	K	E	V	Q	D	F	K	Y	V	G	D	A	S	A	-			
80	W	L	N	F	K	E	V	Q	E	F	K	P	I	G	V	A	P	S	D	S	T	A

CONFIRMATION COPY

Figure 8: Optimal alignment of the complete protein encoded by CcCPI-2 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

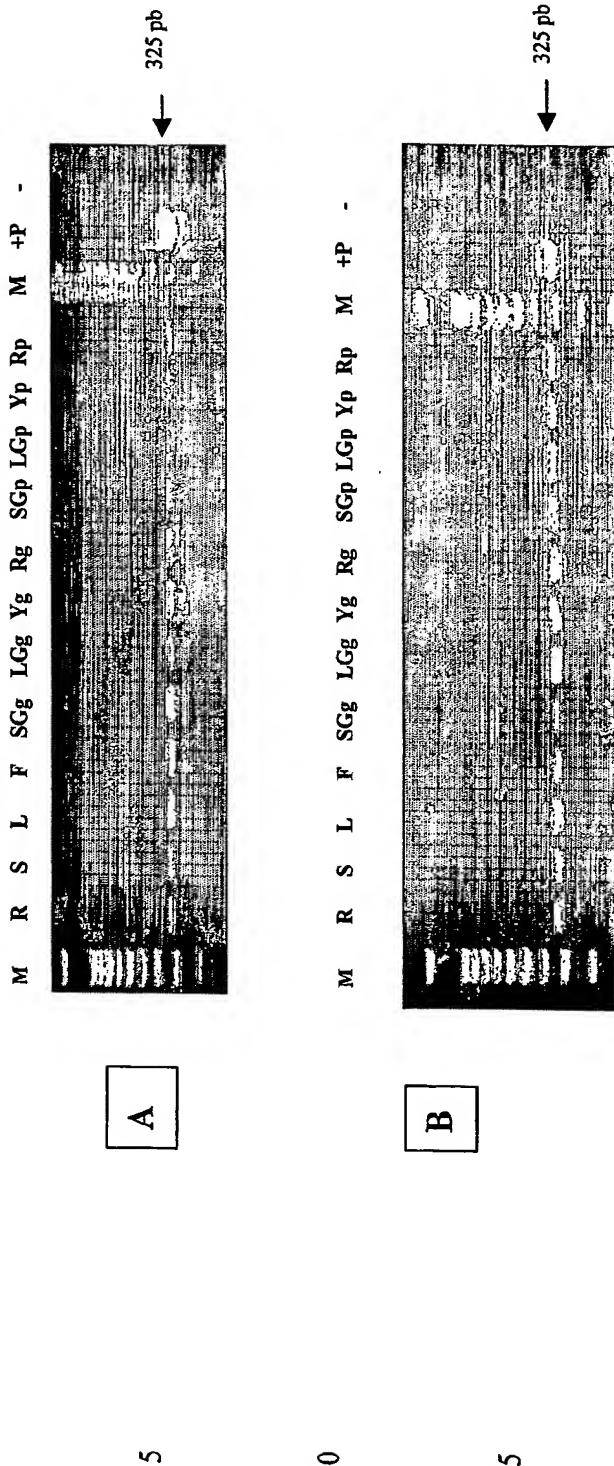


Figure 9: RT-PCR analysis of the expression of CcCPI-2 gene in different tissues of *Coffea arabica* CCCA2 (A) and *Coffea robusta* FRT-32 (B).

Figure 10: Optimal alignment of the complete protein encoded by CcCPI-3 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

CONFIRMATION COPY

1	M A T V A A K S A T A A I G A G Q K N M V G G L S S T Y P P R S S T Y N P K D I P H V I Q	CcCPL4
1	M N Q R F C C L I V I F I S P L L A A G D R K G A L V G G W K P I E D P K E K H V M E	Citrus x paradisi
1	M T S K V V F L L L S L L S L L P L Y A S A A A R V G G W S P I S N V T D P Q V V V	A. thaliana
46	I A Q F : A . V A N Y . N A K A G T T V V W L N V . E Y C F W W I D D T Y Y M L A I K T Q D L T	CcCPL4
46	I G Q F : A V T E Y . N . K Q S K S A L K F E S V E K G E T Q V V S G T N Y R L I L V V K D G P	Citrus x paradisi
45	I G E F A V S F Y N K R S E S G L K F E T V V S G E T Q V V S G T N Y R L K V A A N D G D	A. thaliana
91	- G - H C D V A L V R H I S E S N G T Y S I L K W Y N H N N K .	CcCPL4
91	- S - K F E A V V W E K P W E H - F K S I L T S F K P M V K .	Citrus x paradisi
90	G V S K N Y L A I V W D K P W M K - F R N L : S F E P A N N G R F L	A. thaliana

Figure 11: Optimal alignment of the complete protein encoded by CcCPL4 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

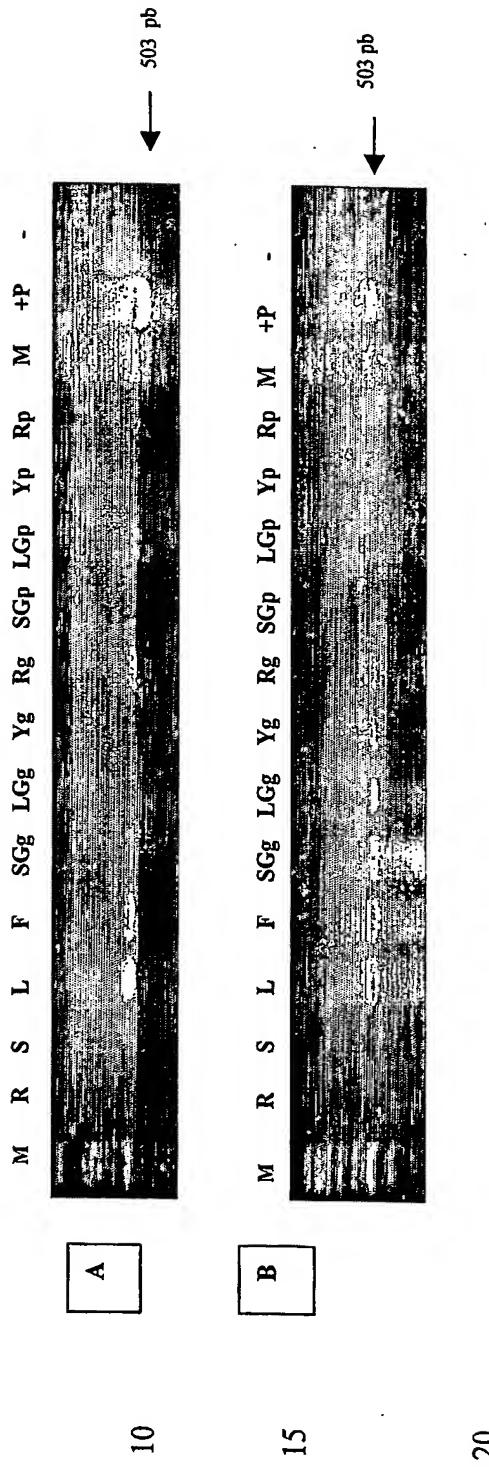


Figure 12: RT-PCR analysis of the expression of CccPPI-4 gene in different tissues of *Coffea arabica* CCCA2 (Panel A) and of *Coffea robusta* FRT-32 (Panel B)

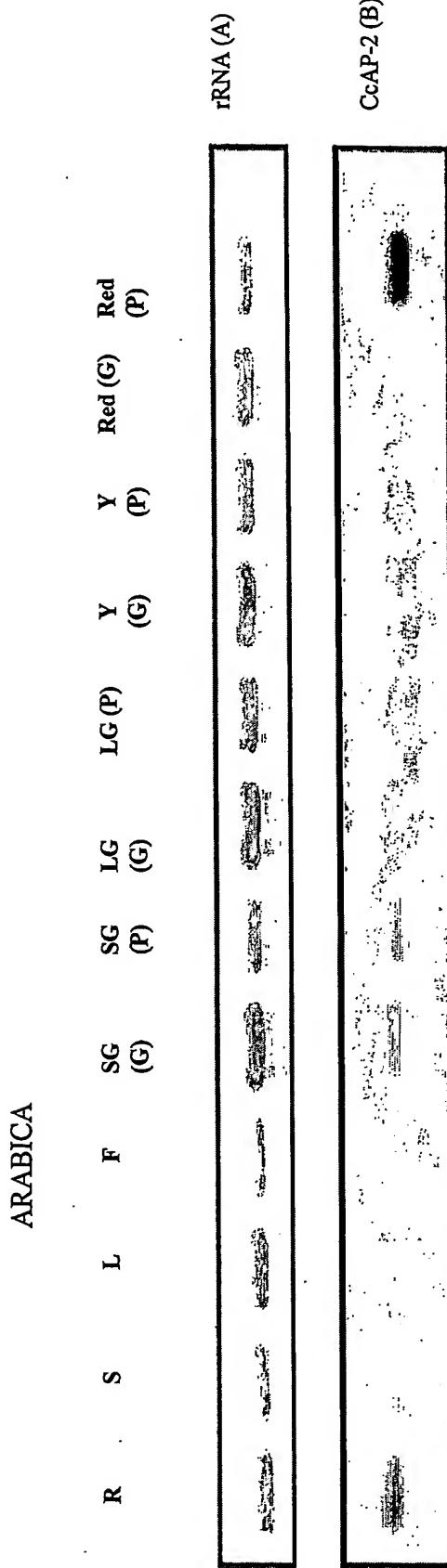


Figure 13: Northern blot analysis of the expression of the aspartic proteinase 2 (CcAP2) gene in different tissues of *Coffea arabica*.

1 gcttacatcttaatccgtatccgtatccgtgaagttcaatccgtcagtcgtcaactaacatgg
 81 agacatacttcgatt ATG AAA ATG GGG AAG GCT TTC CTT TTT GCC GTT GTA TTG GCT GTG ATC
 1 M K M G K A F L F A V V L A V I
 144 TTA GTG GCG GCT ATG AGC ATG GAG ATC ACA GAA AGA GAT TTG GCT TCT GAG GAA AGC TTG
 17 L V A A M S M E I T E R D L A S E E S L
 204 TGG GAC TTG TAC GAA AGA TGG AGG AGC CAT CAT ACT GTT TCT CGA GAC CTT TCT GAG AAA
 37 W D L Y E R W R S H H T V S R D L S E K
 264 CGA AAG CGC TTT AAT GTT TTC AAG GCA AAT GTC CAT CAC ATT CAC AAG GTG AAC CAG AAG
 57 R K R F N V F K A N V H H I H K V N Q K
 324 GAC AAG CCT TAC AAG CTG AAA CTC AAC AGT TTC GCT GAT ATG ACC AAC CAC GAG TTC AGG
 77 D K P Y K L K L N S F A D M T N H E F R
 384 GAA TTC TAC AGT TCT AAG GTG AAA CAT TAC CGG ATG CTC CAC GGC AGT CGT GCT AAT ACT
 97 E F Y S S K V K H Y R M L H G S R A N T
 444 GGA TTT ATG CAT GGG AAG ACT GAA AGT TTG CCA GCC TCC GTT GAT TGG AGA AAG CAA GGA
 117 G F M H G K T E S L P A S V D W R K Q G
 504 GCC GTG ACT GGC GTC AAG AAT CAA GGC AAA TGT GGT AGC TGT TGG GCA TTT TCA ACT GTG
 137 A V T G V K N Q G K C G S C W A F S T V
 564 GTT GGA GTC GAG GGA ATC AAC AAA ATC AAA ACA GGC CAA TTA GTT TCT CTG TCC GAG CAA
 157 V G V E G I N K I K T G Q L V S L S E Q
 624 GAA CTT GTT GAC TGT GAA ACG GAC AAT GAA GGA TGC AAC GGA GGA CTC ATG GAA AAT GCA
 177 E L V D C E T D N E G C N G G L M E N A
 684 TAC GAG TTT ATT AAG AAA AGT GGG GGA ATA ACA ACT GAG AGG CTA TAT CCC TAC AAG GCA
 197 Y E F I K K S G G I T T E R L Y P Y K A
 744 AGA GAT GGC AGC TGT GAT TCG TCA AAG ATG AAT GCC CCT GCT GTG ACT ATT GAT GGG CAT
 217 R D G S C D S S K M N A P A V T I D G H
 804 GAA ATG GTA CCC GCA AAC GAT GAG AAT GCC TTG ATG AAA GCT GTT GCT AAC CAG CCT GTA
 237 E M V P A N D E N A L M K A V A N Q P V
 864 TCA GTA GCT ATA GAT GCG TCT GGC TCT GAC ATG CAA TTT TAT TCA GAG GGT GTA TAC GCT
 257 S V A I D A S G S D M Q F Y S E G V Y A
 924 GGA GAC TCG TGT GGC AAT GAG CTT GAT CAT GGC GTG GCG GTC GTC GGC TAC GGG ACT GCT
 277 G D S C G N E L D H G V A V V G Y G T A
 984 CTT GAC GGT ACT AAA TAC TGG ATA GTG AAG AAC TCA TGG GGA ACA GGA TGG GGA GAA CAG
 297 L D G T K Y W I V K N S W G T G W G E Q
 1044 GGC TAT ATC AGG ATG CAA CGT GGT GTT GAT GCT GCT GAA GGC GGA GTT TGT GGG ATA GCA
 317 G X I R M Q R G V D A A E G G V C G I A
 1104 ATG GAG GCC TCC TAT CCA CTT AAA TTG TCC TCC CAC AAT CCA AAA CCA TCC CCA CCT AAG
 337 M E A S Y P L K L S S H N P K P S P P K
 1164 GAC GAC CTC TAG attgatccctttatatacatatataatattcagtagattcattgaattttagttac
 357 D D L *
 1240 agactacgcgcctcTGaagacttagatcatctctaggatagattatgtatccgtcctgtatggttgaataaac
 1320 aataagtagactaataaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

Figure 14: cDNA sequence and its deduced amino acid sequence of CcCP-4. Lowercase: 5' and 3' non-translated regions; Uppercase: Open reading frame; Bold character: amino acid sequence; *: stop codon

Figure 15: Alignment of the full sequence of the protein encoded by CcCP-4 cDNA with other full-length cysteine proteinases available in the NCBI database

Decoration 'Decoration #1': Shade (with solid black) residues that match KDDL -CCCP4 exactly.0

Figure 16. The full length cDNA sequence CcCP-4 KDDL and the partial cDNA sequence CcCP-4 (KDEL).

5 N K N G K A P I F A V V L A V I L V A A M S M E I T E R D L A S E E S L W D L Y E P W R S H H T V S R D L S E K R K R F N V H T V S R D L S E K R K R F N V H H I H K V N Q K D K P Y CcCP-4 KDDI

1 K L K L N S F A D N T N H E F R E F Y S S K V R K H Y R M L H G S R A N T G F M H G K T E S L P A S V D W R K Q G A V T G V K N Q G K C F S C W A F S T V V V G V E CcCP-4 KDEI

81 G I N K I K T G Q I V S L S E Q E L V D C E T D N E G C N G G L M E H A Y E F I K K S G G G T T E R L Y P Y K A R D G S C D S S K M N A P A V T I D G H E N V P CcCP-4 KDDI

1 G I N K I K T G Q I V S L S E Q E L V D C E T D N E G C N G G L M E H A Y E F I K K S G G G T T E R L Y P Y K A R D G S C D S S K M N A P A V T I D G H E N V P CcCP-4 KDEI

161 A N D E N A L M K A V A N Q P V S V A I D A S G S D M Q F Y S E G V Y A G D S C G N E L D H G V A V V G Y G T A I D G T K Y W I V K N S W G T G W G E Q G Y I R CcCP-4 KDDI

17 A N D E N A L M K A V A N Q P V S V A I D A S G S D M Q F Y S E G V Y F G D S C G N E L D H G V A V V G Y G T A I D G T K Y W I V K N S W G T G W G E Q G Y I R CcCP-4 KDEI

241 M Q R G Y D A A E G G V C G I A N E A S Y P L K L S H N P K S P P K D D I CcCP-4 KDDI

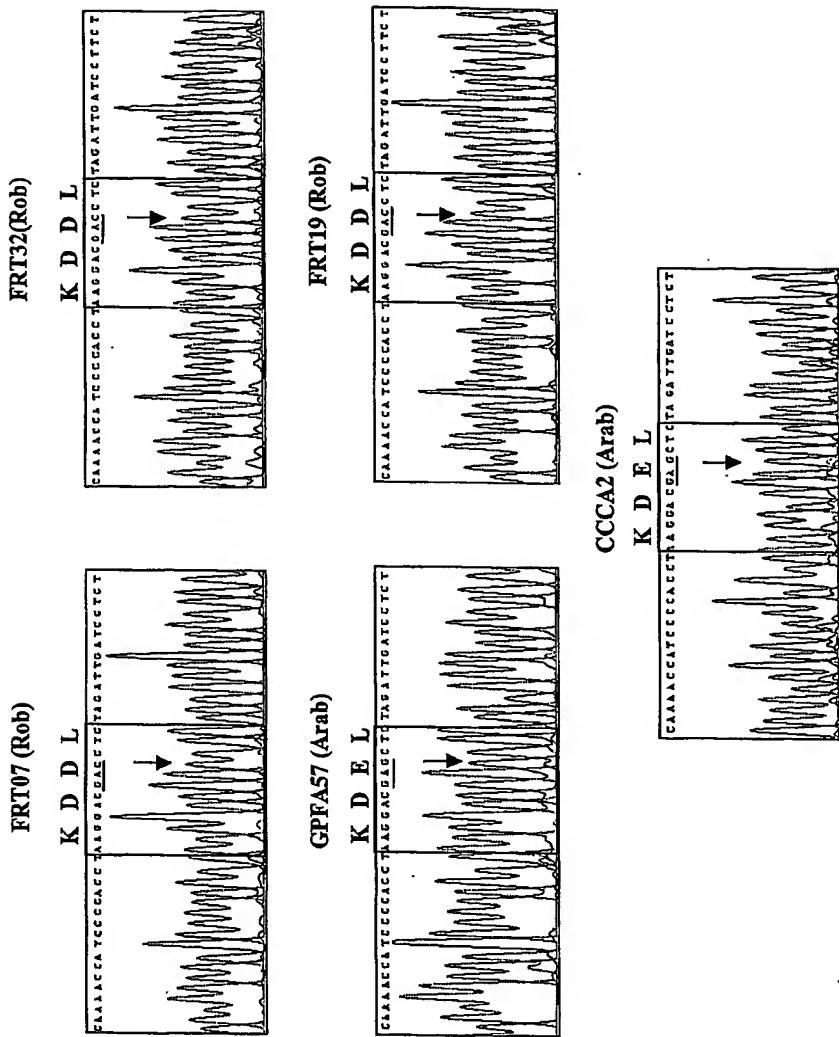
97 M Q R G Y D A A E G G V C G I A N E A S Y P L K L S H N P K S P P K D D I CcCP-4 KDEI

321 M Q R G Y D A A E G G V C G I A N E A S Y P L K L S H N P K S P P K D D I CcCP-4 KDDI

177 M Q R G Y D A A E G G V C G I A N E A S Y P L K L S H N P K S P P K D D I CcCP-4 KDEI

Decoration 'Decoration #1': Shade (with solid black) residues that match CcCP-4 KDDI exactly. □

Figure 17. The complete open reading frame of CcCP-4 (KDDI) and the partial open reading frame of CcCP-4 (KDEL).



5 Figure 18. DNA sequence chromatograms for PCR amplified genomic DNA encoding the KDEL/KDDI region of the CcCP-4 gene.

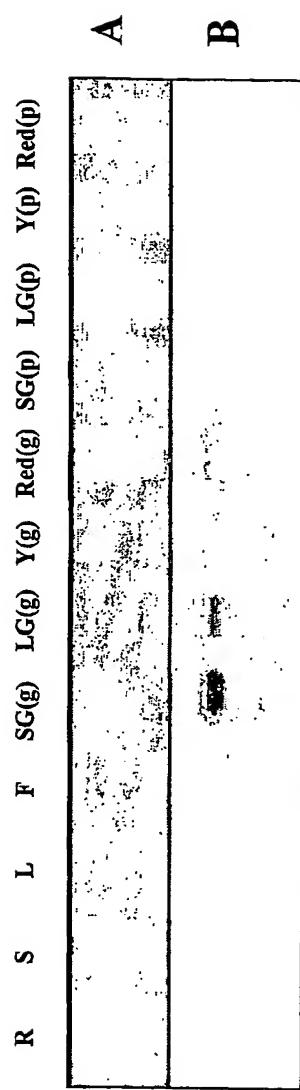


Figure 19. Northern blot analysis of the expression of the Cysteine proteinase CcCP 4 gene in different tissues of *Coffea arabica*.

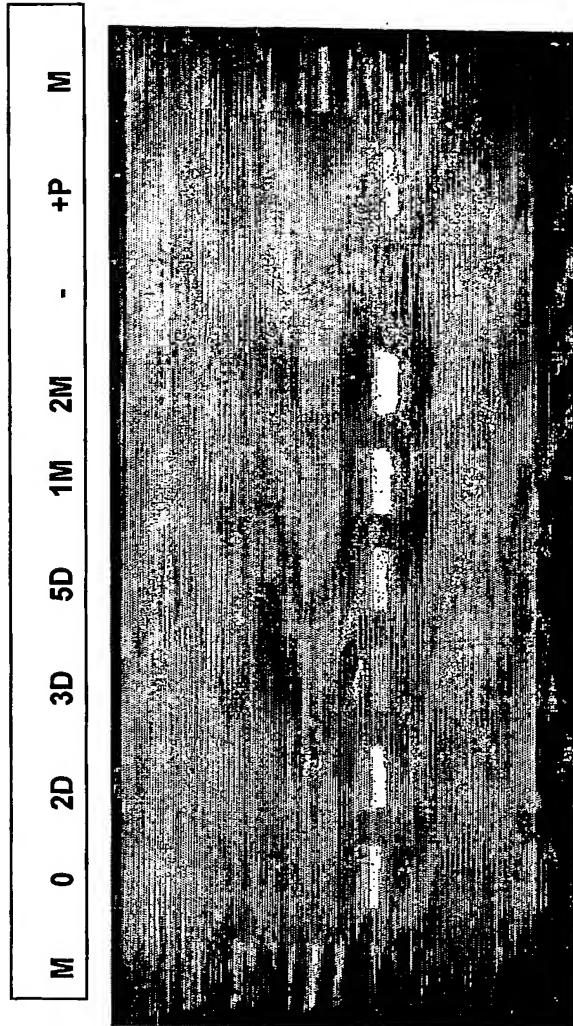


Figure 20. RT-PCR analysis of the expression of CcCP-4 in the whole grain during germination.

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Figure 21: Optimal alignment of the complete protein encoded by CcAP-1 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI.

1 W E R R Y L W A A F V I G A I V C S L F P L P S E G - L K R I S I L K K R P F L D I Q S I R A A K L A H E S T H G A G R K E N - D N N L C C A P 2
 1 W S Q O K H L V T V F C I W A L T C S L L E S S F G - T I L R I G L K K R P F L D I D L D S I N A A R K A R E G L R S V R P M G A H D Q F E I G . m a x
 1 H G R K H L C A A F L I W A V V C T A L P A Y S D N N L L R V G L K K R P F L D I E S T I K A A K G A R L G G K Y G K G V N - - - - - K E , L I . b a t a t a s
 1 D K R K N L W V I F C F C A L I C S C F E S T S A S S D G - L F R I G L K K H R E D U S I K A R V A K L Q D R Y G K H V N G I - E R R S I . e s c u l e n
 1 W G H R K N L W V I F C F C A L I C S C F E S T S A S S D G - L V R I G L K K H R E D U S I K A R V A K L Q D R Y G K H V N G I - Q G L K R F - Q Y S F N . a l a t a N

 66 G S S N - E D I I L P L K N Y L D A Q Y Y G E I G I G T P P Q O K - F T V I F D T G S S S Q . D N V E V G D I V V K D Q . V F E I G L G S L T E V I A K E D G I I - G L G F Q E I A V V C C A P 2
 67 G K S K G E D I I L P L K N Y L D A Q Y Y G E I G I G T P P Q O P E T V F D T G S S S Q . D N V K G S A V V K H 2 D F I E A T H E G S I S I T E L S A K E D G I I - G L G F Q E I S V G . m a x
 65 G D S . D - E G I A V S N N Y L D A Q Y Y G E I S I G S I S G F E S Q D N V K G S A V V K H 2 D F I E A T H E G S I S I T E L S A K E D G I I - G L G F Q E I S V G . m a x
 66 S D S . D - I Y K V P L K N Y L D A Q Y Y G E I G I G S P P Q K F K V I F D T G S S S N L W V P S S K C Y F S I T A C W I H S I K Y O R S K S S S I . e s c u l e n
 65 G D S . D - T D I V Y L K N Y L D A Q Y Y G E I G I G T G S P P Q K F S V I F D T G S S S N L W V P S S K C Y F S V A C Y F H S K Y K S S S S N . a l a t a N

 133 T Y T A I G K S C I R Y G . S . G . S I S G F S Q . D N V E V G D I V V K D Q . V F E I G L G S L T E V I A K E D G I I - G L G F Q E I A V V C C A P 2
 133 T H V R N G T S C K I N N Y G T G S I S G F E S Q D N V K G S A V V K H 2 D F I E A T H E G S I S I T E L S A K E D G I I - G L G F Q E I S V G . m a x
 132 T Y T Q I G K S C S I T Y G S V S I S C F E L S Q D D V Q I G D L I V K U Q V E L I T T R E P S L T E I I A K F D G I I L G L G F Q E I S V G . m a x
 133 T R T D G E S C S I S I R Y G T G S I S G H F S M D N V Q V G D I V V K D Q V F E I E A T R E P S I T E I V A K E D G I I - G L G F Q E I S V G . m a x
 132 T Y T X I G . K . S C E I D Y G S G S I S G F S Q D I V E V G S N L A Y K N Q V F I E A S R E K S I L T F A L A K I F D G I I - G L G F Q E I S V G . m a x

 2001 D N M V P V . W Y N N V D Q G L V D F O V E S F W I L N R . D P N A E D G G E L V F G G V D T N H F K G K H T Y V P V T Q K G Y W Q F K M G D C C A P 2
 2003 E N V A P V W F K M V E Q K L I S I E K V F S I E W L N G D P N A E K G G E L V F G G V D E F K I I F X G N H T Y V P I E X G Y W Q I E X G D G . m a x
 2001 E N V V W V W Y N V D Q G L V D F O V E S F W I L N R . D P N A E D G G E L V F G G V D E F K I I F X G N H T Y V P I E X G Y W Q I E X G D G . m a x
 2000 G N T T P V W V W Y N V G Q G L V K E P V F S I E W L N R . D P N A E K G G E L V F G G V D E F K I I F X G N H T Y V P I E X G Y W Q I E X G D G . m a x
 2000 G D V V P V W Y N V E Q G L V S E K V F S I E W L N R . D P N A E K G G E L V F G G V D E F K I I F X G N H T Y V P I E X G Y W Q I E X G D G . m a x

 2689 F L I G N V S T G E G C E G G C A A I V D S I G T S L I A G F T - V N V T Q I N H A I G A E G V U S T E I C K E I V S Q Y G E L I W D I L V S G C C A P 2
 2721 F E V G G N S T G G V C E G G C A A I V D S I G T S L I A G F T - V N V T Q I N H A I G A E G V U S T E I C K E I V S Q Y G E L I W D I L V S G C C A P 2
 2688 F L I G N S S T G Y G C A V I V D S I G T S L I A G F T - V N V T Q I N H A I G A E G V U S T E I C K E I V S Q Y G E L I W D I L V S G C C A P 2
 2689 F L I G N T S T G Y C A G G C A A I V D S I G T S L I A G F T - V N V T Q I N H A I G A E G V U S T E I C K E I V S Q Y G E L I W D I L V S G C C A P 2
 2688 F L I G N R S T G F C R R G C D A I V D S I G T S L I A G F T - V N V T Q I N H A I G A E G V U S T E I C K E I V S Q Y G E L I W D I L V S G C C A P 2

 3337 V I P D R V C K Q A G L C P L R G A Q H E N A Y I N S V U V D E E N K E E A S V G E S E M C T A C E M A V U V M Q N Q L K Q Q E T K E V V C C A P 2
 3339 V K E P D I C S Q V G I C S S K R H Q S K S A G I E M V T E K E Q - E E L A A R D T P L C S S C Q N L V I W I D V I L V S G C C A P 2
 3336 L R A D Q V C S E L G I C F L N G A W H E S S I K T V V E K E A - E G - N L T S N P L C T C E N A V I W I D V I L V S G C C A P 2
 3336 T I P D Q V C S Q A G J C F L D G S Q H V S S N I R T V V E R E T - E G S S V E A P L C T C E N A V V W M Q N Q L K Q E Q T K E V V L . e s c u l e n
 3336 V Q P D K I C S Q L A C F N - D A Q F I S I G K T V I E R E W R K N K S S V A D F L C T A C E N A V V W I Q Q I R R E V T K E V V N . a l a t a N

 05 L A V N Q L C E S I P . S . P . G . S E S I I D . C . N . S I S T L E N Y S E T I G G K S F E I T I L K E Y V L R I G E G F A , E V C I S G E M P D V C C A P 2
 06 F N Y V N Q L C E S I P . S . P . G . S E S I I S C I N S I S S K M E N I T T I G N K P . E V I I T V Q N Q L K Q K A T K D R V G . m a x
 04 L E Y V N Q L C E K I P S P N G E S A I D C N R I S S E D I T K I D V T P L C T V I K T I G E T I A V C Y S I G E A L D V I L . b a t a t a s
 03 L N Y I N E L C D S I P S P N G E S V I D C S I P Y W P N V - F T I G E K P R L T P E Q V V L R A G E D A M V C L S G F F A L D V N . a l a t a N

Figure 22: Optimal alignment of the complete protein encoded by CcAP-2 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI Nucleotide database. The alignment was performed with the Clustal W program. The alignment is shown with the CcAP-2 sequence at the top. The N-terminal amino acid sequence of CcAP-2 is shown in bold. The alignment shows that CcAP-2 has a high degree of homology with other aspartic proteinases, particularly with the insect aspartic proteinases. The alignment also shows that CcAP-2 has a unique N-terminal sequence compared to other aspartic proteinases.